Biomedical Letters ISSN 2410-955X– An International Biannually Journal

Research article
2022 | Volume 8 | issue 2 | Pages 202-208



In silico structural insight and functional evaluation to predict novel non-coding RNAs of *Enterobacter cloacae complex*

Open

Abbeha Malik, Muhammad Nasir Iqbal*

Department of Bioinformatics, Institute of Biochemistry, Biotechnology and Bioinformatics, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

ARTICLEINFO

Received
June 12, 2022
Revised
July 28, 2022
Accepted
September 02, 2022

***Corresponding Author**
Muhammad Nasir Iqbal

E-mail
nasir.iqbal@iub.edu.pk

Keywords
Non-coding RNAs
Transfer RNAs
Ribosomal RNAs
micro RNAs
intergenic region
minimum free energy

How to Cite
Malik A, Iqbal MN. *In silico* structural insight and functional evaluation to predict novel non-coding RNAs of *Enterobacter cloacae*

Abstract

The non-coding RNA (ncRNA) produces functional RNA molecules instead of encoding proteins, however, the ncRNAs contain information to perform the function. Most genetic information is encoded by proteins while most of the genetic information of mammals and other complex organisms is transcribed into ncRNAs. The current study was designed to predict the ncRNAs in the genome of the *Enterobacter cloacae complex* by employing *in silico* approaches. Various putative ncRNAs were predicted in four different species of *Enterobacter cloacae complex*. Extensive *in silico* analyses were performed and specific promoters were predicted for all the selected ncRNAs. The predicted promoter regions were validated for further analyses. The selected ncRNA was utilized for secondary structure prediction. All the predicted secondary structures were validated through various evaluation tools and secondary structures were observed suitable. All the selected ncRNAs were observed stable and characterized based on hairpin loops, least MFE value and promoter regions. In conclusion, the predicted ncRNAs have the ability to perform stable functions.





Introduction

The non-coding RNAs (ncRNAs) generate functional RNA molecules rather than translated proteins. ncRNAs are unable to encode protein however regulate the associative genes. ncRNAs are involved in several cellular processes including regulation of gene expression, RNA modification and editing [1]. In humans, approximately 98% of the genome can be transcribed and only 2% encodes the protein [2], proving the possibility that a large amount of the genome may encode ncRNA. The predicted numbers of ncRNAs have been still unknown. Usually, ncRNAs may be functional or may not be functional however non-functional ncRNAs are referred to as junk RNA [3]. The functional RNA molecules are components of cellular machines such as ribosomes (ribosomal RNAs), the spliceosome and telomerase.

Enterobacter cloacae complex is a gram-negative, facultatively anaerobic, rod-shaped and non-spore forming bacteria that belongs to the family *Enterobacteriaceae*. Many strains of bacteria are pathogenic. *Enterobacter* species are 0.6-1 µm in diameter and 1.2-3 µm long [4]. 80% of species are encapsulated having an optimal growth temperature of 30 °C. Upon glucose fermentation, the bacteria produce acid. *Enterobacter* species can cause several infections, including cerebral abscess, pneumonia, meningitis and intestinal infections. *Enterobacteriaceae* family is a colonizer of the lower gastrointestinal tract of humans and animals. Plants, animals, or humans can be their hosts.

It is found that particularly ncRNAs are abundant in roles that require highly specific nucleic acid recognition without complex catalysis [5]. The processes that involve ncRNAs are gene regulation, maturation of messenger RNAs (mRNAs), ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), and X-chromosome inactivation in mammals. The genes have been discovered in all kingdoms of life to regulate ncRNAs including microRNA (miRNA) and small interfering RNA (siRNA) in eukaryotic cells [6]. Several regulatory roles involve the bacteria to be acting as antitoxic components in toxin-antitoxin systems by bacterial small RNAs whereas regulatory ncRNAs adjust bacterial physiology with respect to environmental cues. These have also been discovered in several species throughout the bacterial kingdom [7]. The emerging main elements of cellular homeostasis besides microRNA, ncRNAs are PIWI-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), transcribed ultra-conserved regions (T-UCRs) and large intergenic non-coding RNAs (lincRNAs). In addition to microRNAs, tumorigenesis, neurological, cardiovascular, and developmental diseases [8] are found to be caused by the dysregulation of ncRNAs. Bacteria encode an enormous number of small non-coding RNAs (sRNAs) that acts to modulate gene expression at the post-transcriptional level. Many sRNAs often control the expression of outer membrane proteins (OMPs). *Enterobacteria* (*Escherichia coli* and *Salmonella*) are now known to encode at least eight OMP-regulating sRNAs (InvR, MicA, MicC, MicF, OmrAB, RseX and RybB). sRNAs act to show up their functions under diverse growth and stress conditions ncRNAs regulate the associated genes [9].

In silico methods were utilized to predict the novel ncRNAs on basis of general features and common characteristics to predict ncRNA. Over the last decade, progressive improvement has been

observed in the field of computational drug design [10, 11] and bioinformatics and more opportunities are available to understand the biological mechanisms [10]. Numerous biological problems have been resolved by utilizing various bioinformatics approaches [12, 13]. Moreover, structural and functional bioinformatics have efficient techniques to scrutinize and design novel compounds against various disorders including COVID-19 [14-16].

Materials and Methods

ncRNA Prediction

ncRNAs were predicted in genomes of *E. cloacae complex*, for this purpose RNAspace web server was used [17]. The selected server isolates ncRNAs from the genome. Initially, the selected genome was subjected to the RNAspace web server. The species name was utilized along with its strain with default parameters. Homology search parameters were utilized. In comparative analyses, the *Enterobacter sakazaki* organism was selected as the selection of the same organism showed suitable results. FASTA sequence was subjected to BLAST for sequence alignment, CG-sequence as sequence aggregation, RNAz as structure inference for comparative analyses methods. The generated results were prepared in excel sheet format.

Screening of ncRNAs

The manual screening of ncRNAs by deletion of nucleotide sequences with length less than 75 were done as the structures of shorter-length sequences was not considered to be stable. The removal of ribosomal RNAs and transfer RNAs was performed to avoid pseudogenes. On the basis of names given to ncRNAs by RNAspace, repetitions were removed. The removal of the repeats was an important step as the purpose of the current study was to predict structures.

Validation of ncRNAs

The validation of the predicted and screened ncRNAs was performed. Rfam database was used to screen and validate the names of all the selected ncRNAs [18]. The validation confirms the names of already known and unknown genes. Rfam- Xfam database performed ncRNA sequence search. The names of ncRNA sequences were obtained on the basis of bits score, e-value and strand. The ncRNAs that have unknown names were dropped from the experiment.

tRNA search

The presence of tRNAs was validated to avoid the presence of irrelevant tRNAs. tRNAscan-SE [19] was utilized to remove irrelevant tRNAs. ncRNA sequences were subjected in FASTA format for analyses.

Promoter Prediction

Promoter region was predicted for ncRNAs. Promoter 2.0 prediction server [20] was used to predict the promoter regions of ncRNAs. The transcription start sites were predicted for vertebrate Pol II promoters in nucleotide sequences. It has been developed as an evolution of simulated transcription factors that interact with the sequences in promoter regions. FASTA sequences were utilized for the analyses.

Structural characterization of ncRNAs

The secondary structures prediction was performed to visualize the alpha helices and beta pleated sheets of the selected ncRNAs. Mfold web server [21] was used to predict the secondary structures of the selected ncRNAs. RNA folding form option was selected on Mfold and linear form option, size of interior bulge/loop taken as 30, maximum asymmetry of an interior bulge/loop also taken as 30, folding temp was set at 37 °C for the analyses. Numerous structures were generated for detailed analyses.

Functional characterization of ncRNAs

ncRNAs having stable structures are considered suitable with proper functionality. The predicted structures were verified for their stability. MFE value, bulges, hairpin loops, pseudoknots, stem junctions, cross pairing and overlapping were verified. ncRNAs were unable to perform direct function however can regulate the attached genes. In order to check for ncRNA function, associative genes were predicted. BLAST [22] was performed to screen the associative genes. The nucleotide BLAST option of NCBI BLAST was used and stable structured ncRNA sequences were observed. Total score and identity were observed for the final selection of the selected ncRNAs. The predicted associative genes were verified through GenBank. The function of the known gene was verified by using UniprotKB [23].

Results and Discussion

Numerous bioinformatics analyses were performed to screen novel ncRNAs. 304, 197, 239 and 208 ncRNAs were predicted in the intergenic regions of the *E. kobei, E. asburiae, E. cancerogenus and E. hormaechei* genomes respectively. The observed results were analyzed for further analysis. From all the predicted ncRNAs, ribosomal RNAs and transfer RNAs were also observed. Interestingly, numerous known and unknown RNAs were also observed and analyzed. IS1222-FSE, sroE, t44, MicF, ryfA, and LR-PK1 known RNAs were also observed from the generated set of predicted ncRNAs. 3441 ncRNA was predicted in *Burkholderia cenocepacia* strain J2315 (**Fig. 1**). It was observed that the predicted ncRNAs vary from species to species.

From all the predicted ncRNAs, 304, 197, 239 and 208 predicted ncRNAs, ribosomal RNAs, transfer RNAs, repeats and sequences having a length of >75 nucleotides were eliminated and 275, 131, 146 and 142 ncRNAs were retained for further analyses. 3441 putative ncRNAs were predicted in *Burkholderia cenocepacia* strain J2315 and 213 were screened after applying the selected parameters. It was also observed that the number of tRNA, rRNA and shorter sequences may vary from genome to genome and the putative ncRNAs of the selected genome may have a different number of tRNAs and rRNAs. The known and unknown scrutinized sequences (275, 131, 146 and 142 ncRNA sequences) were evaluated and analyzed. The names of all the scrutinized ncRNAs were verified and characterized on the basis of hairpin loop (**Table 1**). The false positive rRNAs and tRNAs were eliminated. Extensive *in-silico* analyses evidenced

	A	
1	>000001|IS1222_FSE|bacteria|kobei|unknown|unknown|+|51836|51953	IS1222_FSE
2	GGCGCTTCAGGTGGCTCTGGGGCGAAAGTACTGACGACAGACCAGAAGCGGGAAGCTGTGGTGTTGATGTGTGATGCGACCGGTCTGTCGCAACGTCGTGCCTGCAGGCTTACAGGTT	
3	>000003|PK-repBA|bacteria|kobei|unknown|unknown|+|68144|68267	PK-repBA
4	AACCCCCTGAAATCTGCAATCAACTTGGCGGAAGGTTCAGATATTCAGGGGGTCATAAAGCAGGCGCGCTGCCTGTGAAGGATTATAACGCATGCACCATATAAAACAACACCCGGCCGCCATA	
5	>000010|GlmZ_SraJ|bacteria|kobei|unknown|unknown|+|4322361|4322551	GlmY_tke1
6	GTAGATGCTCATTCCACCTCTTATGTTCGCCTCAGGGCCTCATAAACTCAGGAATGACGCAGAGCCATTTAATGGTGCTTATCGTCCACAGACAGATGTCGCTTCGGCCTCATCAAACACCATGGACACAACGTT	
7	>000013|LR-PK1|bacteria|kobei|unknown|unknown|+|1974747|1975084	LR-PK1
8	ACCGACGATGGCGACTACCAGGTAAAACTCCGCAGCCTGGTTCGCTTTCTGGAAGAGGGTGATAAAGCTAAGATCACACTGCGTTTCCGCGGTCGTGAGATGGCTCACCAGCAGATTGGTATGGAAGTGCTT	
9	>000014|ryfA|bacteria|kobei|unknown|unknown|+|1399697|1399874	ryfA
10	GCGTCCCTTTCCGCCATCTCGCAAATGGGCACCGATCCAGGGAAAGGATTATCCACAACCGTAATCAGGCACTATTCCGTGCTTGCATCCGCCGAATGATCATCGGTGGTGAGACGGTGGAGCGGTTTTCAGC	
11	>000018|sroD|bacteria|kobei|unknown|unknown|-|2522839|2522923	sroD
12	TTGCGTGACGAAGCCCGCGCCAAAGTAGACAATAAAGTCTGAGCTTTGAGTAAGTCGCCTGACGCCGGTTAGCCGGCGTTTTTTA	
13	>000019|sroE|bacteria|kobei|unknown|unknown|-|3293788|3293880	sroE
14	ATAACGTGACTGGGAAGCGGCTTGCTTCCCGTGTATGATTGAACCCGCAGCGCGCCCGGCAGGTCAGGGTGAGCGCTAAGGGTTCATTTTTAT	
15	>000030|SraG|bacteria|kobei|unknown|unknown|+|3999129|3999293	SraG
16	CTTCTGTGCATCCTCGCGACTAATGACAACCCTAACCCAAACCGGGTAAAGCCTCTCATTAGCCGCGCGAACCTCTGCAACGAAGATCATTCATAGCAACAATACAATAGTTTAGGGTGAATTGCTGCCGTCTGG	
17	>000033|Mg_sensor|bacteria|kobei|unknown|unknown|+|543947|544058	Mg_sensor
18	TTACCGGAGGCAACATGGATCCTGATCCCACCCCTCTCCCCGACGGGAGTTTTCCCGCGTCCCGGTAAGCCAGTTCTCGCTGCCTTGCCAGACGCGTAAGGCAGCGACGTTT	
19	>000034|MicF|bacteria|kobei|unknown|unknown|+|3070405|3070491	MicF
20	CGCTATCATCATTAACTTTTATTTATTACCTTCATTCGGCTTCGAATGACTGTTTACCCCTATTACAACCGGATGCCCTGCATTCGG	

Fig. 1: Top ranked 20 predicted ncRNAs

Table 1: The names of all the scrutinized ncRNAs

Sr #	Name	Rfam	Base
01	IS1222-FSE	IS1222-FSE	117
003	PK-repBA	PK-repBA	123
010	GImZ-Sraj	GImY-tke1	190
013	LR-PK1	LR-PK1	337
014	ryfA	ryfA	177
018	sroD	sroD	84
019	sroE	sroE	92
030	SraG	SraG	164
033	Mg_sensor	Mg_sensor	111
034	MicF	MicF	86
035	Unknown	Unknown	136
040	Unknown	isrK	76
043	DsrA	DsrA	78
044	Unknown	RybB	79
045	SraC_RyeA	RyeB	133
051	Unknown	Unknown	99
053	Unknown	OmrA-B	76

that 259, 131, 146 and 142 sequences have the potential to act as ncRNAs.

The predicted promoters have complex centers to work as transcriptional initiators and initiate the transcription process for the conversion of DNA into RNA. It was observed that there was no promoter attached to the selected refined 259, 131, 146 and 142 ncRNA sequences. The promoter prediction for ncRNAs in *E. coli* resulted in the identification of promoters attached to ncRNAs. Extensive comparative analyses showed that the promoters were attached to ncRNA.

It was observed that all the screened (259 ncRNAs of *E. kobei,* 131 ncRNAs of *E. asburiae,* 146 ncRNAs of *E. cancerogenus* and 142 ncRNAs of *E. hormaechei*) ncRNAs have unstable structures. The stability of the screened structures depends on numerous factors including minimum free energy, number of bulges, number of hairpin loops, cross pairing, overlapping, pseudoknots and stem junctions (**Fig. 2**). The stability of the structures was directly proportional to the least MFE value. The secondary structures were predicted for the screened 4 ncRNAs of *B. cenocepecia* strain J2315. ncRNAs in different genomes were eliminated by having a different number of screened ncRNAs for stable structures.

It was observed that unknown 230, IS1222-FSE and isrK have 4 hairpin-loops, a single nucleotide bulge, three nucleotide bulge, cross-pair, and a least MFE value of -28.60 followed by overlapping.

There were 2 stable structures observed among 259 ncRNA structures of *E. kobei*. The stability of the predicted ncRNAs was verified on the basis of selected parameters. The stable structures satisfied the selected parameters. The stable structures showed hairpin loops, bulges, pseudoknots, stem junctions, and overlapping and cross pairs. The stable structures showed the least MFE values of -62.60 and -73.10 (**Fig. 3A** and **3B**).

Only one stable structure was observed among 131 ncRNAs from scrutinized sequences in *E. asburiae* (**Fig. 3C**). The stable structure showed free hairpin loops, bulges, pseudoknots, stem junctions, overlapping and cross pairs along with least MFE value of -20.90. 2 stable structures were observed in *E. cancerogenus* among 146 ncRNAs. The stable structures showed free of hairpin loops, bulges, pseudoknots, stem junctions, overlapping and cross pairs along with least MFE values of -27.20 and -67.20 (**Fig. 3D** and **3F**). Only one stable structure was observed in *E. hormaechei* among 142 ncRNAs having lowest MFE value of -58.30 (Figure 3E). All the selected stable structures were analyzed for potent functions. ncRNAs have indirect functional capacity.

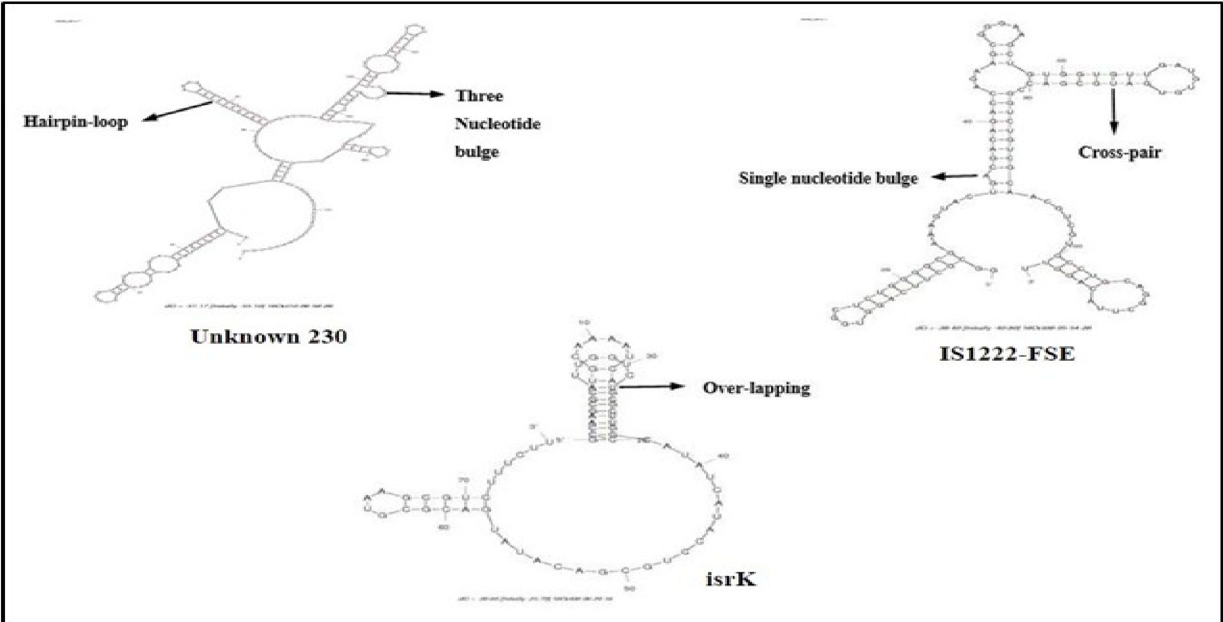


Fig. 2: Non-stable predicted structures

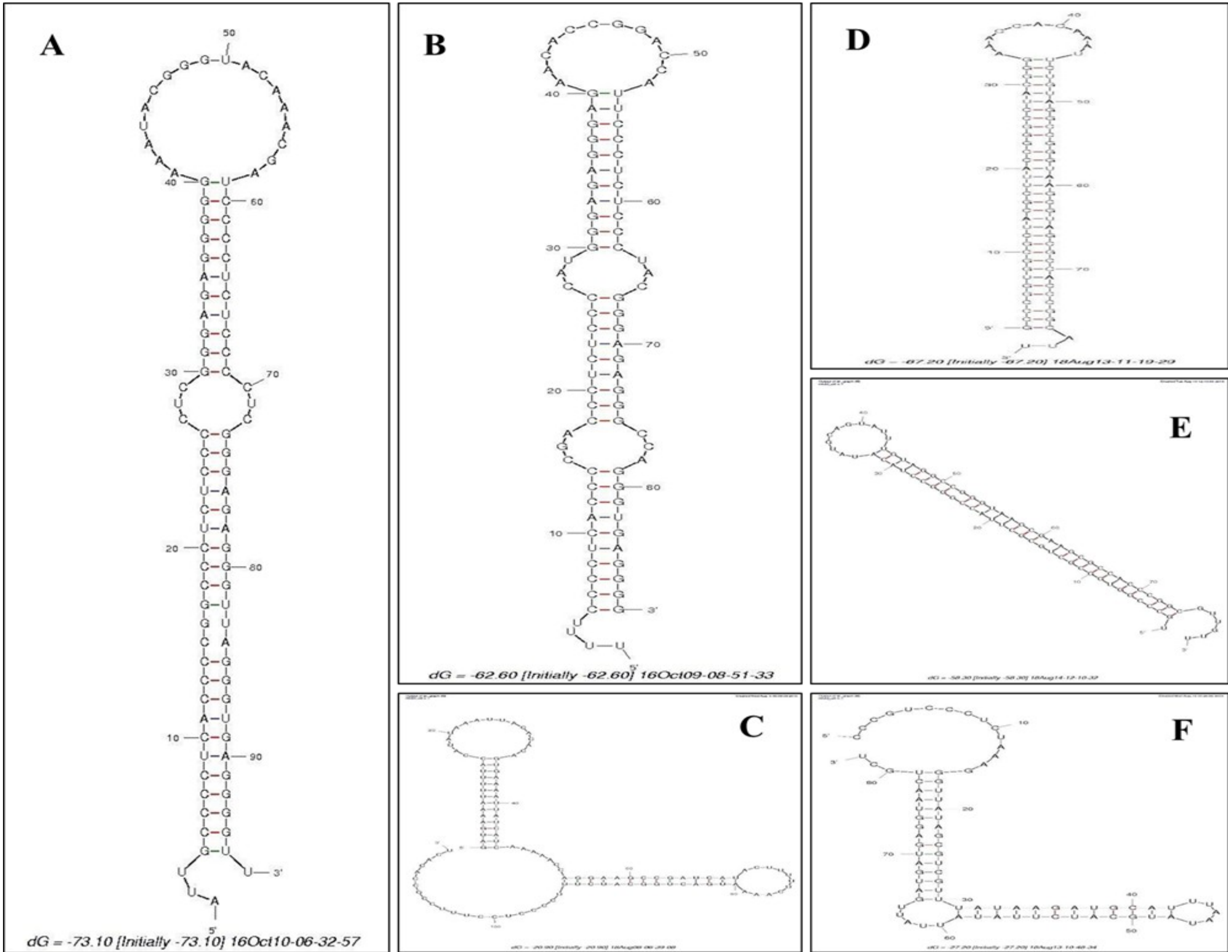


Fig. 3: Stable structures of ncRNAs: (A) *E. Kobie* Unknown 472 (B) *E. Kobie* Unknown 290 (C) *E. Asburiae* Unknown 110 (D) *E. Cancerogenus* Unknown 175 (E) *E. Hormaechei* 097 (F) *E. Cancerogenus* Unknown 065.

Detailed analyses were performed to scrutinize the genes having attached ncRNAs. *Enterobacter kobei* showed the stable structure of ncRNA (unknown290) attached with *ycjD*. The function of *ycjD* was predicted through *in silico* analyses. *ycjD* encoded DNA-cytosine Methyltransferase to improve endonuclease activity. The key function of *ycjD* was to break down the nucleic acid strings and enhance the hydrolysis of ester linkages within nucleic acids (**Table 2**). Endonuclease activity has research applications in marker and primer designing. Moreover it cures viral diseases by breaking viral DNA/RNA. Another stable ncRNA structure (unknown472) was also analyzed and a secondary structure was predicted. The associated genes of ncRNAs of *B. thuringiensis* were cross-verified for further analyses.

Enterobacter asburiae showed one stable structure (unknown110) (**Table 3**). *Enterobacter cancerogenus* showed two stable structures (**Table 4**). *Enterobacter hormaechei* showed only one stable structure (unknown097) and its associative gene (**Table 5**).

Table 2: Functional prediction of stable structure of E. kobei

Sr. #	Name	ncRNA Sequence	Gene	Protein	Function
1	Unknown290	TTTTCCCCTCACCCCGACCCTCTCCCCATG GGAGAGGGAGAACACCGGACCATTCCCTCT CCCTACGGGAGAGGGCCAGGGTGAGGGG	*ycjD*	DNA-cytosine Methyltransfera se	Endonuclease activity
2	Unknown472	ATTGCCCCTCACCCCGGCCCTCTCCCCTCG GGAGAGGGGGAAATACGGGTACAAACGATC CCCTCTCCCCTCGGGAGAGGGTTAGGGTGA GGGGTT	Unknown	Unknown	Unknown

Table 3: Functional prediction of stable structure E. asburiae

Sr. #	Name	ncRNA Sequence	Gene	Protein	Function
1	Unknown11 0	GATGAAAATTTTTACCATATAAATTACACACAGTGAA AATTATCATCAAAAACCAGGAAGCCGATCATACTTTT TCAAAATGACTGGCATCTTTCCCCTCCTTTCCGCCAC ACT	Unknown	Unknown	Unknown

Table 4: Functional prediction of stable structure of E. cancerogenus

Sr. #	Name	ncRNA Sequence	Gene	Protein	Function
1	Unknown065	CCCGTCCCTCTAAAGGGTTATAGCGT CGTTTATAAGATGCATTTAATATGCAT CTTATATTATTGATGATGAGGTAACTG CT	arsC	Arsenate reductase	
2	Unknown175	GCCCGGTGGCGCTACGCTTACCGGG CCTACGGGAAACCACAAATTCTGTAG GCCGGGTAAGCGTAGCGCCACCCGG CATT	dnaA	Chromosomal replication initiator protein	

Table 5: Functional prediction of stable structures of E. hormaechei

Sr. #	Name	ncRNA Sequence	Gene	Protein	Function
1	Unknown097	TGCCCGGTGGCGCTGCGCTTACCGGGC CTACATATGCAGTATTTGTAGGCCGGGT AAGCGAAGCGCCACCCGGCGTTGTT	unknown	unknown	unknown

Enterobacter cloacae complex (ECC) includes common nosocomial pathogens capable of producing a wide variety of infections. Broad-spectrum antibiotic resistance, including the recent emergence of resistance to last-resort

carbapenems, has led to increased interest in this group of organisms and carbapenem-resistant E. cloacae complex (CREC) in particular.

Conclusion

In conclusion, novel ncRNAs were screened and secondary structures were predicted. The function of the scrutinized ncRNAs was also predicted. The novel ncRNAs were characterized on the basis of number of nucleotide, hairpin loops and least MFE values. The genome of *Enterobacter cloacae complex,* showed stable ncRNAs sequences.

Acknowledgement

Authors are thankful to Muhammadan Laboratory of Computational Biology, Pakistan for providing the platform.

Conflict of interest

The authors declare no conflict of interest.

References

[1] Gottesman S. Micros for microbes: non-coding regulatory RNAs in bacteria. Trends Genet 2005;21(7):399-404.
[2] Szymanski CM, Michael FS, Jarrell HC, Li J, Gilbert M, Larocque S, Vinogradov E, Brisson JR. Detection of conserved N-linked glycans and phase-variable lipooligosaccharides and capsules from campylobacter cells by mass spectrometry and high resolution magic angle spinning NMR spectroscopy. J Biol Chem 2003;278(27):24509-20.
[3] Huttenhofer A, Schattner P, Polacek N. Non-coding RNAs: hope or hype? Trends Genet 2005;21(5):289-97.
[4] Paterson DL, Rossi F, Baquero F, Hsueh PR, Woods GL, Satishchandran V, Snyder TA, Harvey CM, Teppler H, Dinubile MJ, Chow JW. In vitro susceptibilities of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections worldwide: the 2003 Study for Monitoring Antimicrobial Resistance Trends (SMART). J Antimicrob Chemother 2005;55(6):965-73.
[5] Eddy SR. Non-coding RNA genes and the modern RNA world. Nat Rev Genet 2001;2(12):919-29.
[6] Gong H, Vu GP, Bai Y, Chan E, Wu R, Yang E, Liu F, Lu S. A Salmonella small non-coding RNA facilitates bacterial invasion and intracellular replication by modulating the expression of virulence factors. PLoS Pathog 2011;7(9):e1002120.
[7] Repoila F, Darfeuille F. Small regulatory non-coding RNAs in bacteria: physiology and mechanistic aspects. Biol Cell 2009;101(2):117-31.
[8] Esteller M. Non-coding RNAs in human disease. Nat Rev Genet 2011;12(12):861-74.
[9] Argaman L, Hershberg R, Vogel J, Bejerano G, Wagner EG, Margalit H, Altuvia S. Novel small RNA-encoding genes in the intergenic regions of Escherichia coli. Curr Biol 2001;11(12):941-50.
[10] Tahir RA, Sehgal SA. Pharmacoinformatics and Molecular Docking Studies Reveal Potential Novel Compounds Against Schizophrenia by Target SYN II. Comb Chem High Throughput Screen 2018;21(3):175-181.
[11] Tahir RA, Hassan F, Kareem A, Iftikhar U, Sehgal SA. Ligand-Based Pharmacophore Modeling and Virtual Screening to Discover Novel CYP1A1 Inhibitors. Curr Top Med Chem 2019;19(30):2782-2794.
[12] Tahir RA, Wu H, Javed N, Khalique A, Khan SAF, Mir A, Ahmed MS, Barreto GE, Qing H, Ashraf GM, Sehgal SA. Pharmacoinformatics and molecular docking reveal potential drug candidates against Schizophrenia to target TAAR6. J Cell Physiol 2019;234(8):13263-13276.
[13] Tahir RA, Bashir A, Yousaf MN, Ahmed A, Dali Y, Khan S, Sehgal SA. In Silico identification of angiotensin-converting enzyme inhibitory peptides from MRJP1. PLoS One 2020;15(2):e0228265.
[14] Balogun TA, Iqbal MN, Saibu OA, Akintubosun MO, Lateef OM, Nneka UC, Abdullateef OT, Omoboyowa DA. Discovery of potential HER2 inhibitors from Mangifera indica for the treatment of HER2-Positive breast cancer: an integrated computational approach. J Biomol Struct Dyn 2021:1-13.
[15] Sajid M, Marriam S, Mukhtar H, Sohail S, Sajid M, Sehgal SA. Epitope-based peptide vaccine design and elucidation of novel compounds against 3C like protein of SARS-CoV-2. PLoS One 2022;17(3):e0264700.
[16] Iqbal MN, Rasheed MA, Awais M, Chammam W, Kanwal S, Khan SU, Saddick S, Tlili I. BMT: Bioinformatics mini toolbox for comprehensive DNA and protein analysis. Genomics 2020;112(6):4561-4566.

[17] Cros MJ, de Monte A, Mariette J, Bardou P, Grenier-Boley B, Gautheret D, Touzet H, Gaspin C. RNAspace.org: An integrated environment for the prediction, annotation, and analysis of ncRNA. RNA 2011;17(11):1947-56.
[18] Kalvari I, Nawrocki EP, Ontiveros-Palacios N, Argasinska J, Lamkiewicz K, Marz M, Griffiths-Jones S, Toffano-Nioche C, Gautheret D, Weinberg Z, Rivas E, Eddy SR, Finn RD, Bateman A, Petrov AI. Rfam 14: expanded coverage of metagenomic, viral and microRNA families. Nucleic Acids Res 2021;49(D1):D192-D200.
[19] Schattner P, Brooks AN, Lowe TM. The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. Nucleic Acids Res 2005;33(Web Server issue):W686-9.
[20] Knudsen S. Promoter2.0: for the recognition of PolII promoter sequences. Bioinformatics 1999;15(5):356-61.
[21] Zuker M. Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res 2003;31(13):3406-15.
[22] Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990;215(3):403-10.
[23] Bastian FB, Chibucos MC, Gaudet P, Giglio M, Holliday GL, Huang H, Lewis SE, Niknejad A, Orchard S, Poux S, Skunca N, Robinson-Rechavi M. The Confidence Information Ontology: a step towards a standard for asserting confidence in annotations. Database (Oxford) 2015;2015:bav043.